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STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS.

IV. The Asexual Larvæ.[1]

J. T. PATTERSON.

I. Introduction.

In 1906 Silvestri[2] reported in his paper on the development of *Litomastix truncatellus* the discovery of a curious type of larva, which he regards as asexual. According to Silvestri, the egg of *Litomastix* produces a thousand or more sexual larvæ and a variable number of these so-called asexual larvæ. The sexual larva undergoes metamorphosis and produces the adult male or female insect. The asexual larva, which dies without undergoing metamorphosis, is characterized by the absence of certain important organs, including the reproductive system.

Silvestri has suggested the possibility that these larvæ may be asexual because of the absence of germ cells. In studying the eggs of two monembryonic species he found that the so-called nucleolus is distributed to the germ cells alone, thus serving as a "keimbahm-determinant." The nucleolus is also present in the egg of *Litomastix*. In the early cleavage stages the nucleolus is included in one of the first four blastomeres, where it breaks down and its contents becomes evenly scattered throughout the cytoplasm. The presence of the nucleolar material retards the subsequent divisions of this blastomere. However, Silvestri was unable to follow the history of this particular cell beyond two divisions, that is, four cells. He suggests that during the course of the development of the polygerm, embryos which receive descendants of the single blastomere possessing the nucleolar

[1] Contribution from the Zoölogical Laboratories of the University of Texas, No. 140.

[2] Silvestri, F., 1906, "Contribuzioni alla conoscenza Biologica degli Imenotteri Parassiti. I., Biologia del Litomastic truncatellus." *Ann. d. Regia Scuola Superiore di Agricoltura di Portici*, Vol. VI., pp. 1–51.

material become sexual larvæ, while those failing to receive such descendants become asexual larvæ.

Various suggestions have been offered to account for the presence of the so-called asexual larvæ in *Litomastix*. Some have suggested that they may not arise from the egg of *Litomastix* at all, but are larvæ belonging to an entirely different species. Thus Wheeler ('10, p. 406)[1] expresses doubt as to Silvestri's interpretation in the following language: "First, the asexual larvæ figured and described by this investigator are suspiciously like certain very young ichneumonid larvæ, and as their development is not satisfactorily traced to the same cell-masses from which the sexual *Copidosoma* larvæ arise, it is not improbable that the two larval forms really belong to two very different parasites. In other words, Silvestri's *Plusia* caterpillars were probably infested with ichneumonid in addition to *Copidosoma* larvæ. Second, I have been unable to find any larvæ of the asexual type in a number of American *Plusia gamma* caterpillars which were heavily infested with *Copidosoma truncatellum*. Third, as in many species of Chalcididæ larvæ of Silvestri's sexual type are able by their own endeavors to break down and assimilate the tissues of their host, it seems improbable that a single species should have developed a peculiar sexless and moribund larva for this particular purpose."

The subject clearly needs reinvestigation. In studying the biology of *Paracopidosomopsis* I have found the same sort of larvæ as those described by Silvestri for *Litomastix*. An opportunity is thus afforded to restudy the question. In the present paper only a brief history of the asexual larvæ will be given. As soon as sufficient material can be secured a detailed account of the cytology and embryology of both types of larvæ will be published.

The material used has been collected here at Austin during the last three years. The species, *Paracopidosomopsis floridanus* Ashmead, is very similar to *Litomastix truncatellus*. It lay its egg in the egg of the common cabbage looper, *Autographa brassicæ*. The parasitic egg develops in the body cavity or tissues

[1] Wheeler, W. M., 1910, "The Effects of Parasitic and Other Kinds of Castration in Insects," *Jour. of Experimental Zoölogy*, Vol. 8, pp. 377–438.

of the *Autographa* caterpillar. By the time the caterpillar reaches the stage for pupation, the larval parasites consume its entire contents, leaving only the skin of the host which later hardens to form the mummified carcass containing the parasitic pupæ. Since the parasites readily emerge in the laboratory the species is very favorable for experimental studies.

Whenever the infection is heavy an abundance of material can be secured from the field. But in order to avoid the possibility of the host egg or larva becoming infected by a species other than *Paracopidosomopsis*, I have used almost exclusively material reared in the laboratory under experimental control. The only exception in this paper is Fig. 12, Plate II.

In all of the experiments the moth eggs have been protected from parasites by having the moths lay their eggs under a bell-jar. The fresh eggs thus obtained are then parasitized. The female parasite has been permitted to make but a single oviposition in each moth egg. The parasitized eggs and the larvæ developing from them have been protected from subsequent infection by keeping them in a closed vessel from which all parasites were scrupulously excluded. Under these conditions of protection, I find that the parasitic egg produces asexual as well as sexual larvæ. These curious larvæ develop irrespective of whether or not the female parasite laying the egg is virgin or impregnated.

From the standpoint of experimental work, the material presents one difficulty. In a previous paper, I have shown that the female, in about two cases out of three, deposits two instead of one egg at a single oviposition. It is frequently difficult, especially in late stages, to determine in a given parasitized caterpillar whether one is dealing with a case involving the product of one or of two parasitic eggs. In early stages this difficulty seldom presents itself, because if two polygerms are present they nearly always lie some distance apart in the tissues of the host.

The age of any stage, referred to in this paper, is based upon the time of oviposition. There is, however, considerable variation in the rate of development of different eggs. Furthermore, temperature greatly influences the rate of development. In August and September about 28 to 30 days elapses between the

laying of the egg and the emergence of the adult insects; whereas in the cooler months of October and November, it takes about 40 to 45 days.

II. The Early Polygerm Stage.

It will be sufficient, for the purposes of this paper, to give only a brief statement concerning the early development of the egg. The freshly deposited egg is a pear-shaped structure, with a large conspicuous nucleus situated toward the anterior or narrower end, and a deeply staining nucleolus lying at the posterior or broad end. Immediately after the egg is deposited, the nucleus undergoes the typical maturation divisions. The first polar body nucleus divides simultaneously with the second maturation division. There is thus produced an egg nucleus and three polar-body nuclei. The former then moves to the broad end of the egg, and whether fertilized or not, produces the cleavage cells. One of the first four blastomeres receives the nucleolus, the presence of which somewhat retards the subsequent divisions of this particular cell. By the time the other three blastomeres have produced about twenty-four cells, the one containing the nucleolar substance has produced but four. Beyond this point its history can not be followed. All of these early blastomeres constitute the embryonic cells. They occupy the posterior two-thirds of the egg.

In the meantime, the polar-body nuclei fuse to form a single polar nucleus, which continues to divide until the protoplasm of the anterior end of the egg is filled with nuclei. The anterior third of the egg thus becomes syncytial in character, and may be called the polar region. In slightly later stages the polar region completely surrounds the mass of embryonic cells, and in stages still more advanced, it forms a nucleated membrane.

About seventy hours after oviposition, the nucleated membrane begins to invade the embryonic cells by the formation of trabeculæ, which divide the embryonic cells into several groups, or primary masses (Fig. 1). During the formation of these masses, or very shortly thereafter, the young polygerm elongates in the direction of the long axis of the egg (Fig. 2). In addition to the nucleated membrane, each primary embryonic mass develops a

second envelope, which lies just inside the nucleated membrane. Apparently, this inner envelope is formed from the peripheral layer of cells of the embryonic mass (Fig. 3). The primary embryonic masses produce secondary masses by fission. A very clear case of division is seen in Fig. 8, on the right.

III. Origin of the Asexual Larvæ.

The first sign of asexual larvæ appears in young polygerms between seventy-five and eighty hours old. One of the youngest stages observed is shown in Fig. 3. An asexual embryo lies at the upper end of the figure, and is distinguishable from the primary masses by two features of its organization. First, the asexual embryo is composed of a large number of relatively small, closely packed cells, whereas a primary mass is composed of a few large cells, showing no definite arrangement. Second, the inner of the two envelopes surrounding the embryo is decidedly hicker than the corresponding membrane of a primary mass. Both of these features become more evident in slightly older stages, such for example, as that shown in Fig. 4. In this specimen the asexual embryo stands in striking contrast to the rest of the polygerm. Furthermore, the constriction of the nucleated membrane, or outer envelope, has all but cut off the embryo from the primary masses. A similar condition is seen in Fig. 5.

At this point it will be well to recall Silvestri's account of the origin of the asexual larvæ in *Litomastix*. The polygerm of *Litomastix*, soon after the nucleated membrane is established, begins to show differentiation into two distinct regions. The anterior region is made up of large and small cells, while the posterior region is composed of small cells only. A constriction appears in the nucleated membrane, which finally separates these two regions. Silvestri names the anterior region the *massa germinigena*, and the posterior the *massa monembrionale*. The latter subsequently differentiates into a single asexual larva. In the course of further development, the massa germinigena gives rise to a few secondary monembryonal masses, which differentiate into asexual larvæ, and to a large number of other masses. This is accomplished by constrictions in the polygerm. The masses continue to multiply by constrictions, and may from

time to time produce a few asexual embryos; but a large majority of them differentiate into sexual embryos.

Some phases of the development of *Paracopidosomopsis* are directly comparable to certain stages of *Litomastix*. The formation of the primary masses and their subsequent mode of multiplication is very similar to the history of the massa germinigena. The chief difference is the early formation of a monembryonal mass in *Litomastix*, for which there is no counterpart in *Paracopidosomopsis*. According to Silvestri the young polygerm always differentiates into a monembryonal mass and the massa germinigena. In the American species a single asexual embryo may be found at an early stage (Figs. 3, 4, 5), but this is by no means the universal rule. In Figs. 6, 7, 8 are shown three well-developed polygerms in which no asexual embryos have as yet appeared. It is not certain whether such polygerms later produce asexual larvæ, although the evidence seems to indicate that at least some of them do. In some cases two or more asexual embryos may develop simultaneously in the very young polygerm. The specimen on the right of Fig. 9 has two such embryos, and Fig. 11 illustrates a similar case. The polygerm shown in Fig. 13 has given rise to four asexual embryos, all in the same stage of development, but situated in different parts of the mass.

These facts indicate clearly that the polygerm of *Paracopidosomopsis* may give rise to one or more asexual embryos at a very early stage of differentiation, but there is no such regularity in the formation of a single monembryonal mass as reported by Silvestri for *Litomastix*.

IV. Dissociation of the Polygerm.

We have already mentioned the fact that the primary masses divide to produce the secondary masses. An early phase of this process is seen in Fig. 6. The primary mass at the extreme left and the one lying on the lower side has each undergone several divisions. There are at least four secondary masses visible in the median section of each primary center. In a still younger stage, Fig. 8, the primary mass on the right is just completing the first division. At this stage of development each secondary mass consists of one or more large central cells and an indistinct

peripheral layer of smaller cells. Surrounding the mass is a thin envelope formed from the trabeculæ of the nucleated membrane. The large central cells are clearly the embryonic cells proper.

The multiplication of secondary masses to produce other masses continues for a long period, at the close of which the final products of division develop into embryos. However, during the course of development, usually early in the period of multiplication, the polygerm fragments or dissociates, the secondary masses becoming widely scattered throughout the body cavity of the caterpillar. The point at which dissociation takes place varies greatly in different cases. It may occur at the close of the third day, or it may be delayed until as late as the tenth or eleventh day. Indeed, in some cases, the polygerm does not completely dissociate until the larvæ are ready to be set free in the body cavity of the host. Apparently, the dissociation is in part controlled by the relation of the polygerm to the host tissues. If the polygerm lies free in the body cavity, or in loose tissue, dissociation will occur very early; but if it is imbedded in fat or other dense tissue, the dispersal of the secondary masses may be greatly delayed.

The polygerm on the left of Fig. 9, although only seventy-seven hours old, has already begun to fragment. Several secondary masses lie some distance from the main body of the polygerm. Two of these appear in the photograph. The polygerm seen in Fig. 13 is ninety-five hours old, and lies free in the body cavity. Dissociation is well advanced, and secondary masses are widely dispersed. In addition to the secondary masses, it has formed four asexual embryos at as many different points. The polygerm in Fig. 11 is eleven days old. It was dissected from the fat tissue of the host. It contains a large number of secondary masses, some of which are beginning to break loose from the main mass, and two conspicuous asexual embryos. These few cases will serve to show the variation in the time at which dissociation begins.

V. Development and Fate of the Asexual Larvæ.

While some polygerms produce asexual embryos at a very early stage (Figs. 3, 4, 5, 9, 13), nevertheless the majority of such

embryos do not appear until after dissociation has taken place. The production of asexual larvæ in a given polygerm is not confined to a single period of development, but is a continuous process, extending from the third to about the fifteenth day. Sections of practically every polygerm from twelve to fourteen days old will show asexual individuals in various stages of development, from young embryos to fully developed larvæ.

The method of their development in the American species is very similar to that for *Litomastix* as described by Silvestri. The main difference is that they frequently appear in groups, instead of arising singly from certain of the secondary masses. This is also true for some of the early cases (Figs. 9, 11). Fig. 14 is a section passing through a group of asexual embryos imbedded in adipose tissue. In the upper right-hand corner of the figure is a group of secondary masses about on the point of developing into sexual embryos. They are easily distinguishable from the asexual embryos by their comparatively small size. Several other series show this same condition. Single embryos or small groups of two or three do, however, appear in some of the series.

After the period of multiplication of secondary masses is closed, that is in stages fourteen or fifteen days old, the body cavity of the host contains many groups of asexual embryos (Figs. 15, 16, 12) as well as groups of young asexual larvæ. In each of two series a group of ten or twelve individuals was found. There are also found free asexual larvæ (Figs. 20, 21, 22), and some that are on the point of being set free from the capsule (Fig. 18). That single asexual larvæ may develop in conjunction with a group of sexual embryos is clearly evident in two instances. In Fig. 17 a young asexual larvæ is closely associated with a group of sexual embryos, and in Fig. 19 a large asexual larvæ, just freed from its capsules, is still attached to a mass of embryos.

The frequent appearance of asexual embryos or larvæ in groups suggests that, like the sexual embryos, the individuals of a group have a common origin, probably arising through the division of a single secondary mass.

Since one cannot follow the course of development of a single egg, but must depend upon series of sections, it is impossible to determine whether every polygerm eventually produces asexual

larvæ. Dissections of a large number of infected caterpillars, twelve to fifteen days old, reveal the presence of asexual larvæ in nearly every case. It is probable that in a few cases no asexual larvæ are developed. It is also difficult to determine the exact number of asexual larvæ produced by a given egg. This is due to the fact that these larvæ are formed continuously from ths third to the fifteenth day, and those first developed degenerate before the last ones appear. The smallest number of larvæ found was one, the largest fifteen. Careful dissections of a series of infected caterpillars reared in the laboratory gave the following data on asexual embryos and larvæ:

Ser. 251, a.	Twelve	days	old—	6 free larvæ; 5 embryos.
Ser. 252, a.	Fourteen	"	"	—1 degenerating larva; 1 embryo.
Ser. 274, j,	"	"	"	—5 free larvæ; 5 embryos.
Ser. 264, c.	Fifteen	"	"	—4 free larvæ.
Ser. 275, i.	"	"	"	—4 free larvæ; 4 degenerating larvæ.
Ser. 251, b.	Sixteen	"	"	—1 degenerating larva; 1 embryo.
Ser. 252, b.	Eighteen	"	"	—No asexual larvæ or embryos.

These data indicate that not more than twelve to fifteen asexual larvæ are produced by a single egg. They also show the period in which these larvæ are developed. The youngest stage containing free larvæ was twelve days old, while the oldest was sixteen days. The asexual larvæ have all degenerated by the eighteenth day. This conclusion has been verified by an extensive study of infected caterpillars from the field. No asexual larvæ have been found in caterpillars whose size indicated that they were eighteen days or older.

The general appearance of the fully developed asexual larva is shown in Figs. 20–22. I have not worked out the details of their organization, but it is clear from a study of entire mounts and sections that the reproductive system is absent. Whether, as in *Litomastix*, the respiratory system, circulatory system, and malpighian tubules are absent, I have not determined.

The asexual larvæ apparently do not live more than a very few days after being set free in the body cavity, probably not over three days. Free larvæ first appear on the twelfth day, and degenerating specimens are found on the fifteenth day. The last larvæ escape from their envelopes on the sixteenth day, and disappear on the eighteenth day. Without exception all asexual

larvæ degenerate. The beginning of degeneration is marked by a foreshortening and twisting of the body (Figs. 23–25). The larva then becomes immobile and soon disintegrates (Fig. 26). So far as I can observe, the larvæ perform no function. There is no evidence that they break down the tissues of the host in preparation for assimilation by the sexual larvæ. They disappear at least a week before the sexual larvæ become free agents.

Whatever may be the underlying cause of the development of these non-viable larvæ, there is certainly no doubt as to the fact that they are formed from the egg of *Paracopidosomopsis*. This fact is established by the results obtained from controlled experiments in which all other parasitic species have been excluded, and by tracing their origin back to the beginning of their development in the young polygerms.

AUSTIN, TEXAS,
June 20, 1918.

Explanation of Plate I.

Fig. 1. A median longitudinal section of a young polygerm. The egg from which this polygerm developed was about 73 hours old. The egg was laid by a fertilized female. × 385.

Fig. 2. From the same lot as the preceding, but slightly more advanced in development. × 379.

Figs. 3–10. All from sections of larvæ on the point of hatching. About 77 hours old. The eggs were laid by fertilized females. × 389.

Fig. 3. Longitudinal section of polygerm, showing a young asexual embryo at upper end.

Fig. 4. Same as preceding, with asexual embryo at lower end.

Fig. 5. A polygerm with asexual embryo on the right.

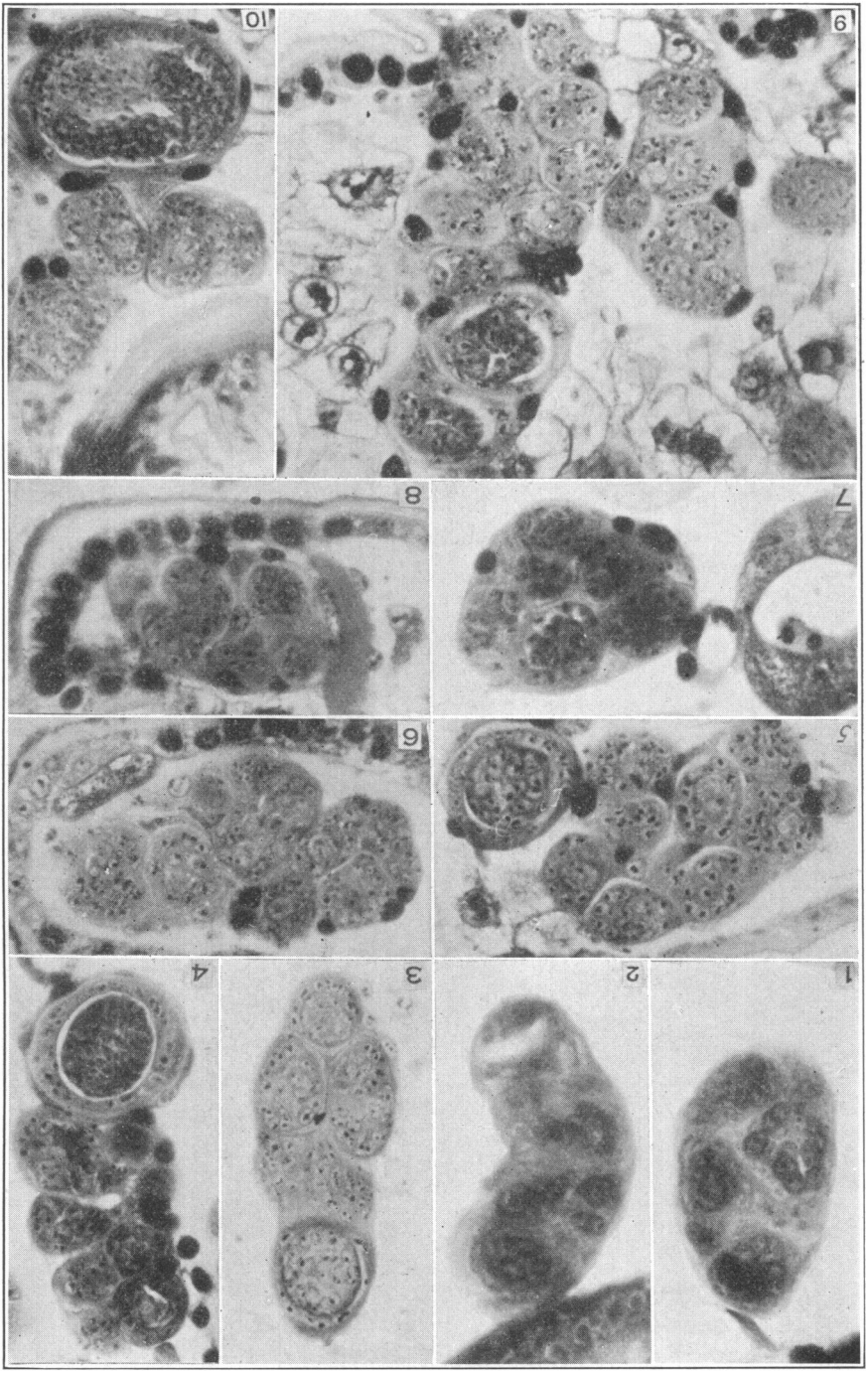
Fig. 6. A polygerm showing no axesual embryo.

Fig. 7. Transverse section of polygerm, showing no asexual embryo.

Fig. 8. Same as preceding. On the right is seen an embryonal mass in the act of dividing.

Fig. 9. Two polygerms in tissue of host. The one on the left has undergone partial dissociation, while the one on the right has not yet dissociated. The latter shows two asexual embryos at the upper end.

Fig. 10. Part of a dissociated polygerm, showing a well-developed asexual embryo and four embryonal masses.


10
9
8
7
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4
3
2
1

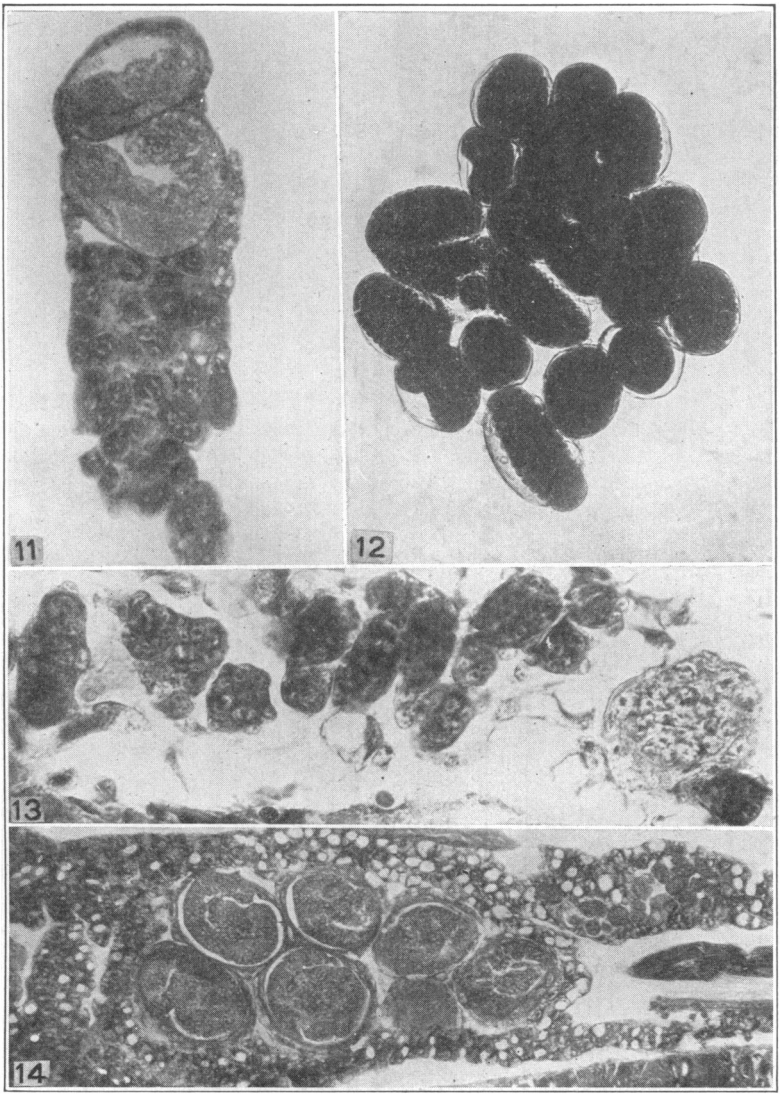
Explanation of Plate II.

Fig. 11. Longitudinal section of an advanced polygerm removed from the adipose tissue of a 12-mm. caterpillar. The polygerm is 11 days old. The egg was laid by a fertilized female. Two large asexual embryos are shown in the upper end of the figure. × 148.

Fig. 12. A mass of sexual embryos. One of several masses removed from the body cavity of a 30 mm. caterpillar taken from the field. × 41.

Fig. 13. Part of a longitudinal section of a polygerm in the body cavity of 5.5 mm. caterpillar. The polygerm has undergone almost complete dissociation. One of the four asexual embryos present in this polygerm is seen at the right. Egg laid by fertilized female. 95 hours old. × 490.

Fig. 14. Part of a dissociated polygerm, showing a group of seven asexual embryos, and a group of embryonal masses in the upper right-hand corner. All are embedded in fat tissue. The polygerm is 9 days and 23 hours old. × 136.



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EXPLANATION OF PLATE III.

FIGS. 15, 16, 19, 22–26 are from eggs (or egg) laid by an unfertilized female. The specimens were taken from the body cavity of a half-grown caterpillar 15 days after the eggs had been deposited. The body cavity contained 4 normal and 4 degenerate asexual larvæ, in addition to many masses of sexual embryos. Figs. 17, 18, 20 and 21 are from eggs (or egg) laid by a fertilized female. The specimens were removed from the body cavity of a 20-mm. caterpillar, 14 days after the eggs had been deposited. There were found in the body cavity of the caterpillar 6 free asexual larvæ and 4 still enclosed in their membranes, besides many masses of sexual embryos.

FIGS. 15, 16. Two masses of sexual embryos. × 100.

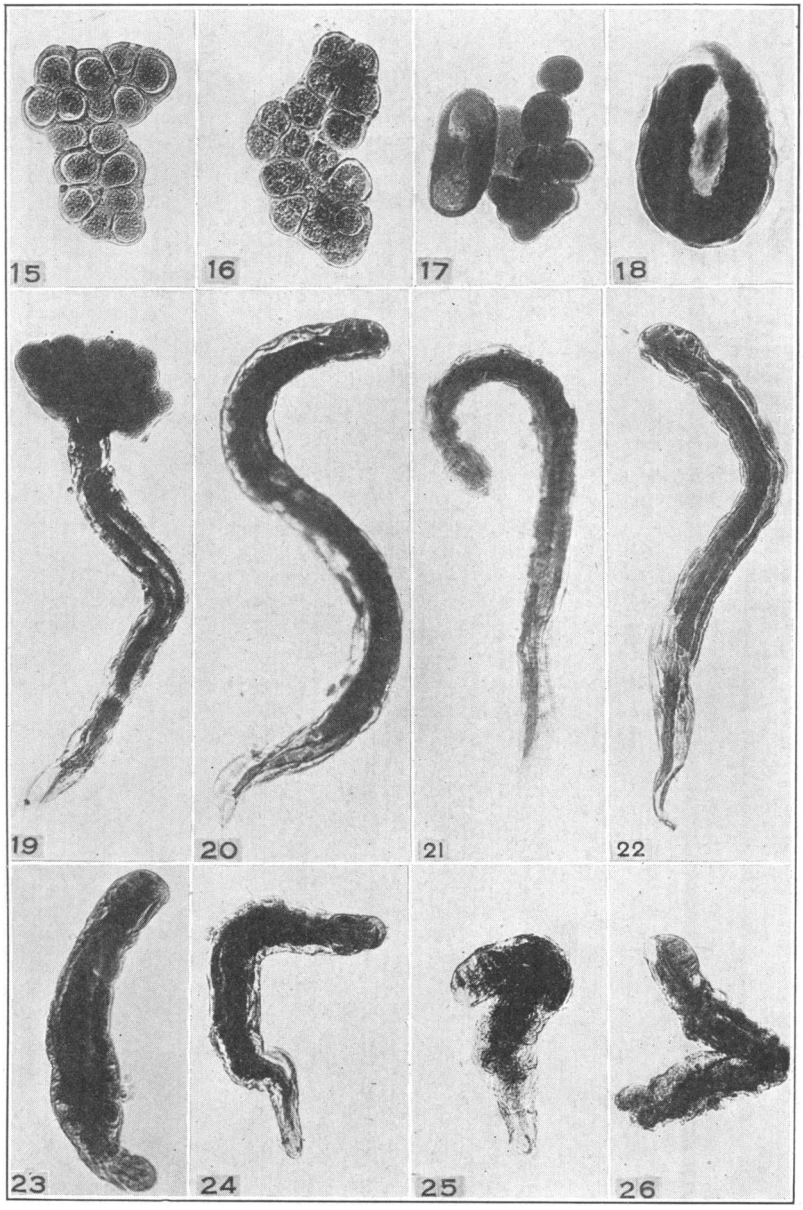
FIG. 17. Mass of sexual embryos with asexual embryo in capsule. × 100.

FIG. 18. Asexual larvæ in capsule. × 100.

FIG. 19. Asexual larva free from capsule but still adhering to mass of sexual embryos. × 88.

FIGS. 20–22. Three asexual larvæ. × 88.

FIGS. 23–26. Four degenerating asexual larvæ. × 88.



J. T. PATTERSON.